

Plant Cytokinesis: Motoring To The Finish Dispatch

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Cytokinesis in plant cells involves a microtubule-containing structure, the phragmoplast, which guides the formation of new cell walls. Recent studies have identified kinesin-like proteins that appear to play a variety of roles in plant cytokinesis.

Cytokinesis in most plant tissues is achieved through the formation of a new cell wall between the daughter nuclei from a recently completed mitosis [1]. A cytoskeletal structure called a phragmoplast acts as a scaffold to guide the movement of Golgi-derived vesicles containing cell-wall components to its equator, where fusion of the vesicles initiates the formation of a new cell wall (called a cell plate while under construction). Each half of the barrel-shaped phragmoplast contains a dense array of short, parallel microtubules oriented with their 'plus' ends interdigitating at the phragmoplast equator (Figure 1A). It has long been thought that vesicles are driven along phragmoplast microtubules to the site of cell plate formation by a plus-end-directed motor protein. A number of kinesins have been localized to phragmoplasts, but until recently no good candidates for a vesicle-translocating motor had been identified [2]. Two recent studies have now identified candidates for such a motor, which may be one and the same protein [3,4].

To complete cytokinesis, the phragmoplast expands laterally until the cell plate reaches the parental wall and plasma membrane, while microtubules disappear from the center of the phragmoplast where the cell plate was initiated earlier (Figure 1A). A further study, published recently in *Current Biology* [5], shows that another kinesin-like protein is required for the proper completion of cytokinesis, and implicates this kinesin in the coordination of cell-plate formation with removal of microtubules from the center of the expanding phragmoplast.

Ultrastructural analysis of cell-plate formation in the developing endosperm (part of the seed) reported by Otegui *et al.* [3] has provided new evidence for the involvement of a kinesin in vesicle transport to the cell plate. In the endosperm, multiple rounds of mitosis without cytokinesis initially create a syncytium, which is later cellularized as cytokinesis almost simultaneously partitions each nucleus into a separate cell [6,7]. As illustrated in Figure 1B, syncytial-type cell-plate formation in the endosperm is initiated by 'mini phragmoplasts' of microtubules that radiate from the surfaces of neighboring nuclei. Otegui *et al.* [3] used

dual-axis high-voltage electron tomography to analyze high pressure-frozen/freeze-substituted samples of developing *Arabidopsis* endosperm, which permitted the construction of high-resolution, three-dimensional images revealing features of cell-plate formation that had not been seen previously. One such feature is the linkage of most vesicles in the phragmoplast to microtubules via a pair of kinked, rod-shaped structures (Figure 1B). These connecting elements resemble the kinesin molecules seen in earlier studies linking latex beads incubated with kinesin to microtubules, and linking membrane-bounded organelles to microtubules in neurons [8].

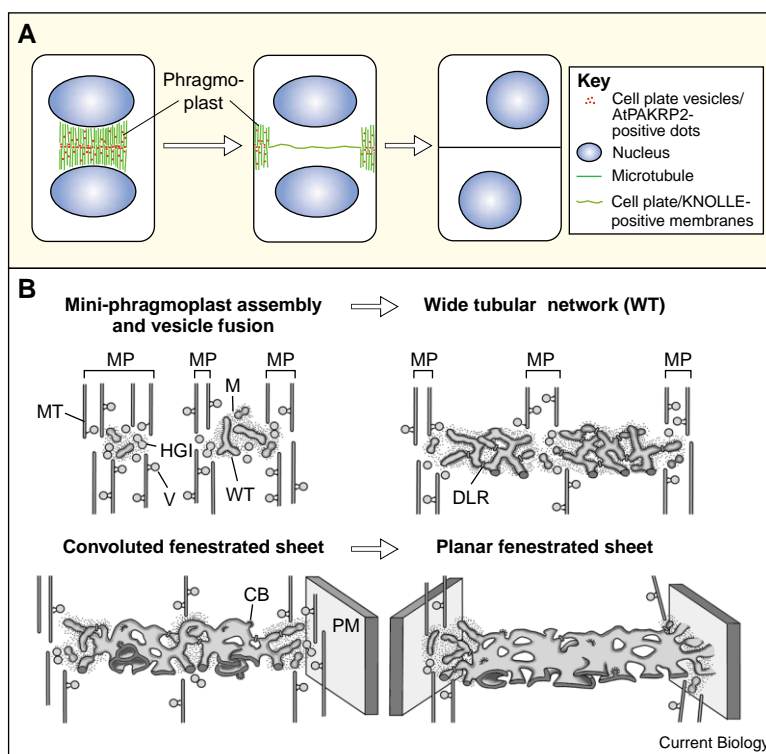
The work of Otegui *et al.* [3] also revealed a number of other interesting features of syncytial-type cell plate formation, one of which provides new insights into the role of dynamin-like proteins in plant cytokinesis. Dynamin polymers wind around membrane tubules and utilize the energy derived from GTP hydrolysis to constrict or sever them. The dynamin-like soybean protein phragmoplastin can polymerize *in vitro* [9] and is localized to cell plates at an early stage of their formation [10]. A role for phragmoplastin in cell-plate formation is also implied by the observation that its overproduction interferes with the normal completion of cytokinesis [11]. The *Arabidopsis* phragmoplastin homolog ADL1 is also cell-plate-localized [12,13]. Although analysis of *adl1* mutants showed that ADL1 is not required for cytokinesis, this is probably due to functional redundancy as ADL1 belongs to a family of closely related proteins [13]. Thus, although phragmoplastin and ADL1 have been implicated in cell-plate formation, their roles remain poorly understood.

As illustrated in Figure 1B, membrane fusion events at the site of cell-plate formation in *Arabidopsis* endosperm lead to the formation of wide tubules, which subsequently fuse with each other to form a labyrinthine wide tubular network. The tubules of this network are periodically constricted by rings consisting of one or two-turn spirals (Figure 1B). Otegui *et al.* [3] showed that ADL1 is present in these rings by immunogold labeling with an anti-ADL1 antibody. Their observations imply a role for ADL1 in the constriction of wide tubules, proposed to help maintain the tubules for a prolonged period of time prior to their consolidation into a 'fenestrated sheet' and ultimately a complete cell wall (Figure 1B).

In their recent study, Lee *et al.* [4] identified an *Arabidopsis* kinesin-like protein, AtPAKRP2, which may correspond to the kinked, rod-shaped structure which Otegui *et al.* [3] observed linking vesicles to phragmoplast microtubules in developing endosperm. AtPAKRP2 was initially identified from its sequence as a novel kinesin-like protein that does not belong to any of the established kinesin subfamilies, and was therefore designated as an outgroup kinesin-related protein. As expected for a kinesin, AtPAKRP2 binds to microtubules in an ATP-dependent manner, although

Figure 1. Cytokinesis in plant cells.

(A) In most plant tissues, cytokinesis is achieved through the action of a phragmoplast formed between daughter nuclei immediately following mitosis (only microtubule and vesicle components of the phragmoplast are shown; see [1] for a more comprehensive review of phragmoplast structure and function). The phragmoplast and cell plate are initiated in the center of the cell, and then expand laterally to complete formation of a new cell wall while microtubules are removed from the center of the phragmoplast. (B) Stages of syncytial-type cell-plate formation in *Arabidopsis* endosperm, emphasizing membrane fusion and remodeling events. Cell-plate formation is initiated within a multinucleate syncytium through the action of mini phragmoplasts (MP). Vesicles (V) are linked to phragmoplast microtubules (MT) via kinked, rod-shaped structures resembling kinesins. Hourglass-shaped intermediates (HGI), formed through initial fusion of individual vesicles, elongate to form wide tubules (WT) surrounded by a fuzzy, ribosome-excluding matrix (M). Subsequent fusion of wide tubules leads to the formation of a wide tubular network, in which tubules are periodically constricted by dynamin-like rings (DLR). Subsequent consolidation of the wide tubular network into a fenestrated sheet is associated with the disappearance of dynamin-like rings, removal of excess membrane via clathrin-coated buds (CB), and fusion of cell plate membranes with the plasma membrane (PM). (Adapted from [3].)



microtubule-translocating activity has not yet been demonstrated. Antibodies specific for AtPAKRP2 showed that it is localized specifically to the phragmoplast and is not found associated with any other microtubule-based structures.

As illustrated in Figure 1A, AtPAKRP2 is localized to puncta within the phragmoplast, with a distribution similar to that of vesicles. Consistent with the possibility that AtPAKRP2 is vesicle-associated, it sediments from cell extracts at between 10,000 and 100,000 x g. The strongest evidence that AtPAKRP2-labeled puncta in the phragmoplast are vesicles is the observation that they are absent in cells treated with brefeldin A, which disrupts the Golgi and thereby eliminates Golgi-derived vesicles. Functional studies will be needed to confirm a role for AtPAKRP2 in vesicle translocation to the cell plate. Electron microscope level immunolocalization to phragmoplast vesicles could also help to further establish a role for AtPAKRP2 in vesicle translocation.

Several other kinesins or kinesin-like proteins, in addition to AtPAKRP2, have been localized to phragmoplasts, though most are found in other microtubules arrays as well. Functional studies have implicated four of these kinesins in the initial formation of phragmoplasts or the maintenance of phragmoplast microtubule organization, while the functions of the rest remain unknown [2]. The recent work of Strompen *et al.* [5] has led to the identification of a new kinesin-like protein of *Arabidopsis* which is required for cytokinesis. A role for the *HINKEL* (*HIK*) gene in cytokinesis

was indicated initially by the observation that *hik* mutant embryos have a high frequency of incomplete cell walls and multinucleate cells. *HIK* is a kinesin-like protein with a putative amino-terminal motor domain and belongs to a plant-specific kinesin subfamily. *HIK* and its orthologs in other plants form a distinct subgroup among plant-specific kinesins which does not include known phragmoplast-localized kinesins. Consistent with a role in cell division, the *HIK* gene appears to be expressed in a cell-cycle-regulated manner in developing embryos.

Detailed examination of abnormal cell divisions in *hik* mutant embryos provided some intriguing clues to *HIK*'s role in cytokinesis. Antibodies specific for the syntaxin *KNOLLE*, which localizes specifically to membranes of the cell plate where it promotes the fusion of cell plate vesicles [12] (Figure 1A), were used to show that *KNOLLE*-positive cell plates are formed in cytokinetic cells of *hik* mutant embryos. Thus, *HIK* does not appear to be involved in transport of vesicles to the cell plate. Moreover, the organization of microtubules in *hik* phragmoplasts is essentially normal, indicating that *HIK* is not involved in phragmoplast assembly or maintenance of phragmoplast microtubule organization. However, the loss of microtubules from the center of the phragmoplast that normally accompanies its lateral expansion (Figure 1A) fails to occur in *hik* mutant cells. At the time when *hik* mutant cell plates reach the full width of the dividing cell, the associated phragmoplasts span the entire cell diameter instead of being restricted to a ring associated with

the periphery of the cell plate. Thus, one possible role for HIK in cytokinesis is to stimulate the disassembly of phragmoplast microtubules at an appropriate stage of cell-plate formation, facilitating in an unknown way the proper maturation of the cell plate. Alternatively, HIK may play some other role in cell-plate development, such as delivering an essential cell-plate component. In that case, abnormal progression of cell-plate development in *hik* mutants may be responsible for the failure of microtubules to be removed from the center of the phragmoplast at the normal time. Localization of HIK protein in dividing cells and ultrastructural analysis of cell plate formation in *hik* mutant cells could both help to further clarify the role of HIK in cytokinesis.

AtPAKRP2 and HIK join several other kinesins previously implicated in plant cytokinesis [2]. Analysis of the completed *Arabidopsis* genome sequence shows that it encodes 61 kinesins [14], though interestingly, no dyneins [15]. The functions of most of these kinesins have yet to be explored, and many more may turn out to play roles in cytokinesis. Ever-improving tools for reverse genetic analysis, including PCR-based identification of T-DNA or transposon insertions into genes of interest and gene silencing through RNA interference, can now be combined with methods for protein localization and biochemical analysis of protein function to accelerate progress toward the goal of understanding the varied contributions of kinesins to plant cytokinesis.

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